12-2-93

MRID No. 427131-03

DATA EVALUATION RECORD

1. **CHEMICAL:** Acetochlor.
Shaughnessey No. 121601.

2. **TEST MATERIAL:** Acetochlor; Batch/Lot/NBR No. QUE-9001-1482-T; 92.07% active ingredient; a brown liquid.

3. **STUDY TYPE:** 72-3. Mollusc 96-Hour Flow-Through Shell Deposition Study. Species Tested: eastern oyster (*Crassostrea virginica*).

4. **CITATION:** Reed, D. and J.P. Swigert. 1992. Acetochlor: A 96-Hour Shell Deposition Test with the Eastern Oyster (*Crassostrea virginica*). Project No. 139A-132. Prepared by Wildlife International Ltd., Easton, MD. Submitted by Acetochlor Registration Partnership. EPA MRID No. 427131-03.

5. **REVIEWED BY:**

Louis M. Rifici, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Louis M Rifici
Date: 5/21/93

6. **APPROVED BY:**

Rosemary Graham Mora, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Rosemary Graham Mora
Date: 5/21/92

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature: William Robert 10/29/93
Date: H T Craven 12/2/93

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a mollusc shell deposition study. The 96-hour EC_{50} value of 3.82 mg a.i./l (based on mean measured concentrations) classifies acetochlor as moderately toxic to eastern oysters. The NOEC was 2.5 mg a.i./l mean measured concentration.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

1

11. MATERIALS AND METHODS:

A. Test Animals: Eastern oysters (*Crassostrea virginica*) were obtained from a commercial supplier in Pasadena, MD. The oysters were held under test conditions for at least 10 days prior to test initiation. They were supplied with unfiltered natural seawater and their diet supplemented with algae (*Thalassiosira* sp.). During holding, the temperature was 21.4-23.0°C, the salinity was 21-25 parts per thousand (ppt), and the pH was 7.5-8.3.

An impartially-selected sample of 20 oysters had an average length of 40 mm (31-48 mm). Immediately prior to test initiation, 4-11 mm of shell periphery was removed from each oyster using a motorized grinder.

B. Test System: A continuous-flow diluter was used. The diluter was preconditioned with the test material for approximately 29 hours prior to testing. Each test chamber received approximately 1 l of test solution per oyster per hour. The test chambers were Teflon®-lined, 56-l polyethylene aquaria filled with 12.6 l of test solution. The test solution depth was approximately 7 cm.

The aquaria were indiscriminately positioned in a temperature-controlled water bath designed to maintain 22 ±1°C. The laboratory environment was maintained on a 16-hour daylight photoperiod with a light intensity of 323 lux. Thirty-minute dawn and dusk simulations were used.

Unfiltered natural seawater, collected at Indian River Inlet, DE, was aerated and diluted with well water before use as test dilution water. The salinity of the dilution water was 17-25 ppt and the pH was 8.2-8.3 during the 4-week period immediately preceding the test.

One stock solution was prepared for each of the five concentrations. The first stock (53.4 mg/ml) was prepared by dissolving the test material in dimethylformamide (DMF). Aliquots of this stock were diluted with DMF to prepare the four additional stocks. The stocks were delivered to the diluter mixing chambers.

C. Dosage: Ninety-six-hour, flow-through toxicity test. Five concentrations (0.25, 0.50, 1.0, 2.0, and 4.0

mg/l), a solvent control, and a dilution water control were chosen for the definitive test. The concentration of solvent in the solvent control and exposures was 0.07 ml/l.

D. **Design:** Twenty oysters were impartially selected and distributed to each aquarium, one aquarium per concentration. To supplement the oyster diet, an algal suspension (*Thalassiosira psuedonana*) was added to the test solutions.

Observations of mortality and clinical signs of toxicity were made daily. At the end of the test, the length of the longest finger of new shell growth on each oyster was measured to the nearest 0.05 mm. The dissolved oxygen concentration (DO), salinity, and pH were measured in each test chamber on days 0, 2, and 4. The temperature was measured in each chamber at the beginning and end of the test. The temperature of the control vessel was recorded continuously.

Test solution samples were collected from each test chamber at 0 and 96 hours. The samples were analyzed for acetochlor using gas chromatography.

E. **Statistics:** Dilution water control and solvent control deposition were compared using a t-test. Shell growth inhibition in each treatment group was expressed as a percentage of the mean growth in the solvent control. The 96-hour EC_{50} value and 95% confidence interval were determined using the percent inhibition data and the binomial probability method.

12. **REPORTED RESULTS:** The mean measured concentrations were 0.29, 0.64, 1.3, 2.7, and 5.2 mg/l (Table 1, attached).

There were no mortalities or observations of sublethal responses during the test. The 1.3 and 5.2 mg/l test solutions were cloudy on day 0 due to the presence of oyster sperm in the test chambers. Oyster shell growth in the dilution water control and solvent control averaged 5.21 and 4.56 mm, respectively (Table 4, attached). Compared to the solvent control growth, only shell growth at 5.2 mg/l was inhibited (Table 3, attached).

During the test, the DO ranged from 6.2 to 7.0 mg/l (>60% of saturation). The pH values ranged from 8.0 to 8.3 and the temperature was 21.5-22.5°C. The salinity was 24-25 ppt.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

The 96-hour EC_{50} value was 4.2 mg/l with 95% confidence limits of 2.7 and 5.2 mg/l. The no-observed-effect concentration (NOEC) was 2.7 mg/l.

Quality Assurance and Good Laboratory Practice Statements were included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards set forth in 40 CFR Part 160. The dates and types of quality assurance audits were reported. Characterization of the test material was the responsibility of the sponsor.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedures were generally in accordance with the guidelines with the following deviations:

In this study, the flow rate of the test solution was 1 l/oyster/hour. According to the protocols recommended by the SEP (APHA, 1981 and Anonymous, 1976), each oyster should receive a minimum of 5 L of flow-through test solution per hour. However, the above method is considered acceptable because a supplemental diet was added and the control oysters met the minimum new shell growth requirement.

B. Statistical Analysis: The reviewer used mean measured concentrations of active ingredient (Table 1, attached) and EPA's Toxanal computer program to determine the 96-hour LC_{50} as 3.82 mg a.i./l (printout 1, attached). The 95% confidence interval could not be calculated but was estimated to be 2.5-4.77 mg a.i./l.

Solvent control shell deposition was determined to be significantly lower than dilution water control deposition using a t-test. The shell growth data were analyzed using one-way analysis of variance and Dunnett's test (Toxstat version 3.3). The NOEC was 2.5 mg/l relative to the solvent control (printout 2, attached).

C. Discussion/Results: This study is scientifically sound and meets the guideline requirements for a mollusc shell deposition study. The 96-hour EC_{50} value of 3.82 mg a.i./l (based on mean measured concentrations) classifies acetochlor as moderately toxic to eastern oysters. The NOEC was 2.5 mg a.i./l mean measured concentration.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** N/A.

(3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 05-17-93.

5

ACETOCHLOR

Page_____ is not included in this copy.

Pages 6 through 8 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Printout #1

RIFICI ACETOCHLOR EASTERN OYSTER 05-14-93

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
4.77	100	85	85	9.536742E-05
2.5	100	0	0	5.765915
1.22	100	0	0	2.012253E-02
.59	100	0	0	2.012253E-02
.27	100	0	0	9.536742E-05

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 3.823503

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

****

PRINTOUT # 12

427131-03, acetochlor, oyster shell deposition
File: a:42713103.oy Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies
Data PASS normality test. Continue analysis.

Bartletts test for homogeneity of variance
Data PASS homogeneity test at 0.01 level. Continue analysis.

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	4.5600	CALCULATED t VALUE =	-2.0464
GRP2 (BLANK CRTL) MEAN =	5.2075	DEGREES OF FREEDOM =	38
DIFFERENCE IN MEANS =	-0.6475		

TABLE t VALUE (0.05 (2),40) = 2.021** SIGNIFICANT DIFFERENCE at alpha=0.05
TABLE t VALUE (0.01 (2),40) = 2.704 NO significant difference at alpha=0.01

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	298.346	49.724	41.052
Within (Error)	133	161.098	1.211	
Total	139	459.444		

Critical F value = 2.18 (0.05,6,120)
Since F > Critical F REJECT Ho:All groups equal

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent contrl	4.560	4.560		
2	dilution contrl	5.207	5.207	-1.860	
3	0.27	4.898	4.898	-0.970	
4	0.59	4.605	4.605	-0.129	
5	1.22	4.995	4.995	-1.250	
6	2.5	4.570	4.570	-0.029	
7	4.77	0.685	0.685	11.134	*

Dunnett table value = 2.32 (1 Tailed Value, P=0.05, df=120,6)

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	solvent contrl	20			
2	dilution contrl	20	0.807	17.7	-0.647
3	0.27	20	0.807	17.7	-0.338
4	0.59	20	0.807	17.7	-0.045
5	1.22	20	0.807	17.7	-0.435
6	2.5	20	0.807	17.7	-0.010
7	4.77	20	0.807	17.7	3.875